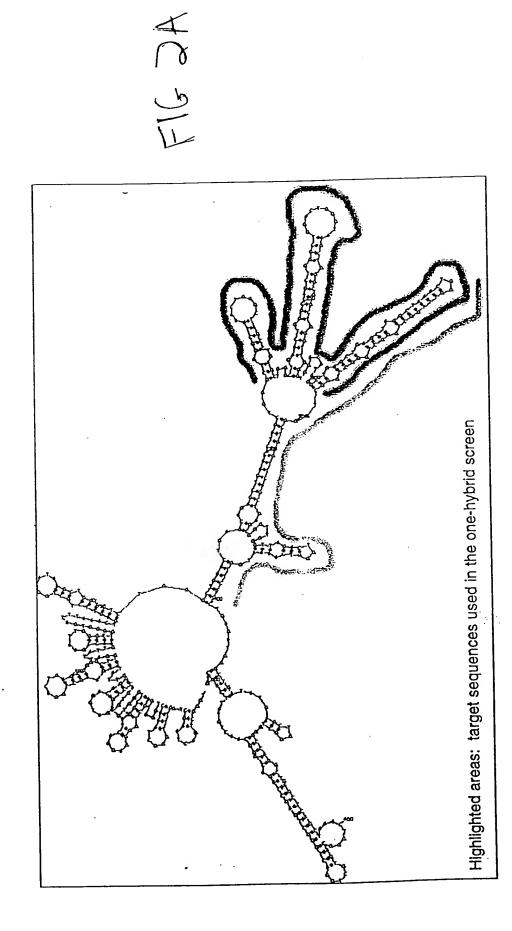
FIGURE 1A

CTCGAGGACAGTGACCTGGGAGTGAGTACAAGGTGAGGCCACCACTCAGGGT GCCAGCTCCAAGCGGGTCACAGGGACGAGGGCCATCAGGAGGCCCT GCACACACATCTGGGACACGCGCCCCCGAGGGCCAGTTCACCTCAGTGCGCCT CATTCTCCTGCACAAAAGCGCCCCCATCCTTTCTTCACAAGGCTTTCGTGGAAG CAGAGGCGTCGATGCCCAGTACCCTCTCCCTTTCCCAGGCAACGGGACCCCAA GTTTGCTGACTGGGACCACCAAGCCACGCATGCGTCAAGAGTGAGAGTCCGG GACCTAGGCAGGGGCCCTGGGGTTGGGCCTGAGAGAGAAAGAGAACCTCCCCC AGCACTCGGTGTGCATCGGTAGTGAAGGAGCCTCACCTGACCCCCGCTGTTGC TCAATCGACTTCCCAAGAACAGAGAGAAAAGGGAACTTCCAGGGCGGCCCGG GCCTCCTGGGGGTTCCCACCCCATTTTTAGCTGAAAGCACTGAGGCAGAGCTC CCCCTACCCAGGCTCCACTGCCCGGCACAGAAATAACAACCACGGTTACTGAT CATCTGGGAGCTGTCCAGGAATTC

Low energy DNA folding of the SE region



Appendix E

FIGURE 2B

- 1 GCTGGGCTAA ACTGGGCTAG CCTGAGCTGG GCTGAACTGG GCTGCTGGGC
- 51 TGGACTGGGT AAGCTGGGCT GAGCTGGGTT GGGTGGAAAT GGGCTGAGCT

FIGURE 2C

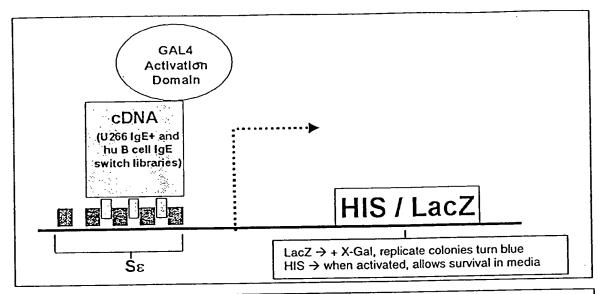
- 1 GGTTTGGCTG GGCTGGGCTG GGCTGGGCTG AGCGGGTTGG
- 51 GTTAGACTGG GTCAAACTGG TTCAGC

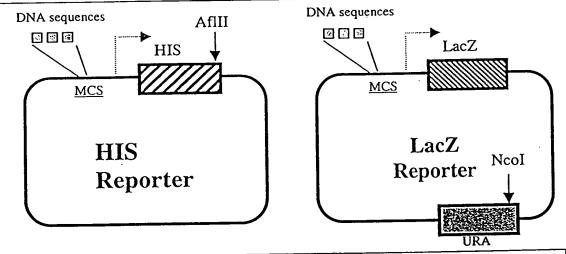
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Appendix F

Yeast One-Hybrid Screening





One hybrid reporter vectors

DNA sequences of interest are inserted into the multiple cloning sites (MCS). The enzyme used to linearize the vector is shown with a solid arrow. Dashed arrows indicate the transcription of the reporter gene.

J 5 1 1

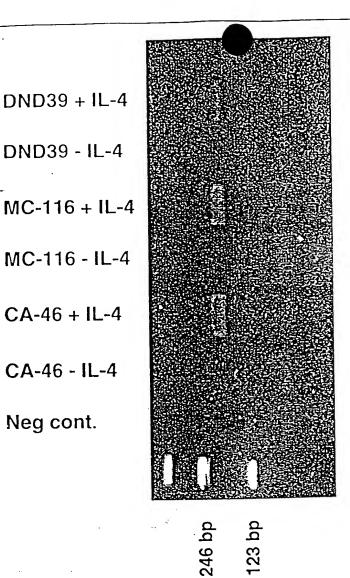
IgM+ B cell lines: CA-46, MC-116 and DND39 IL-4 Induction of Germline ϵ mRNA in the

specific for the germline ϵ (predicted size ~ 200 bp). performed using primers Cells were incubated for 48 hrs in 300 U/ml of hexon and the 5'-end of IL-4. RT-PCR was the E CH1 exon

MC-116 - IL-4 CA-46 + IL-4 CA-46 - IL-4 Neg cont.

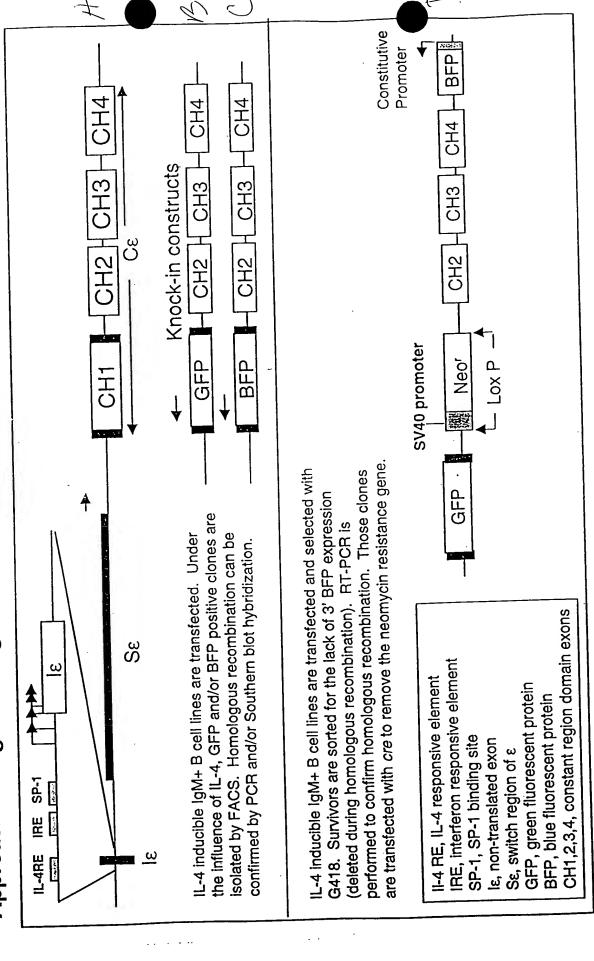
DND39 + IL-4

DND39 - IL-4



Appendix G

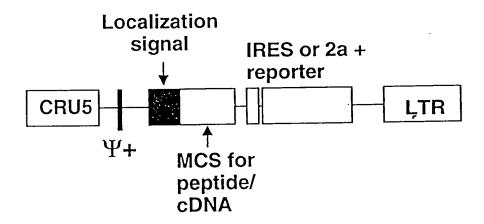
Approaches to generate germline ϵ promoter knock-in reporter cell lines



Appendix A

F166

Appendix I Rigel Base Vector



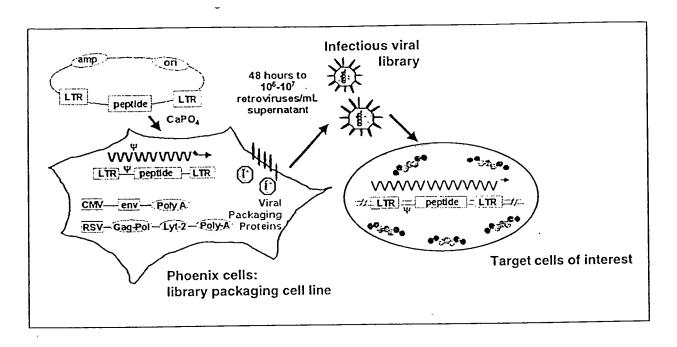
All components are cassetted for flexibility

CRU5, modified LTR
LTR, long terminal repeat
ψ+, packaging signal
Localization signal: nuclear, cell membrane, granular
MCS, multiple cloning site
IRES, internal ribosome entry site
2a, self-cleaving peptide

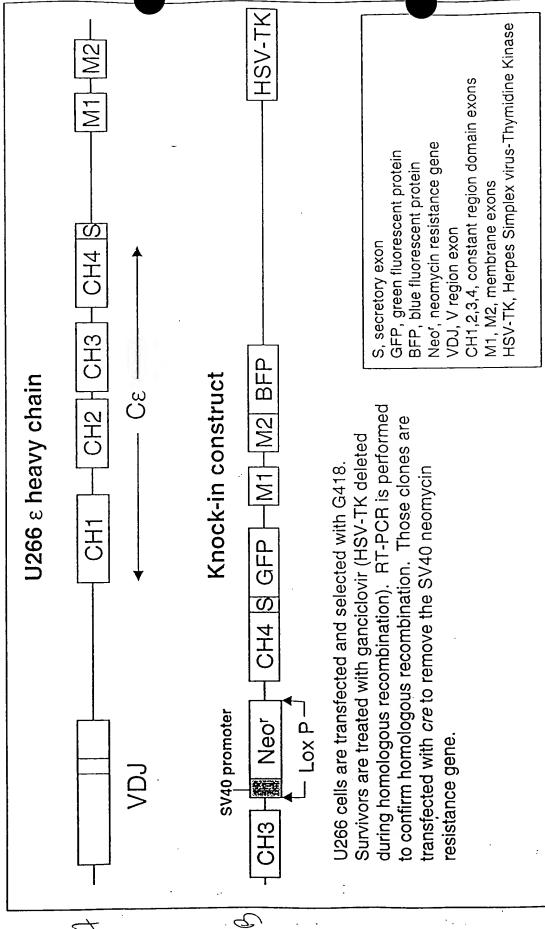
F167

Appendix H

Protocol for Transfection of Phoenix Cells and Infection of Nonadherent Target Cells

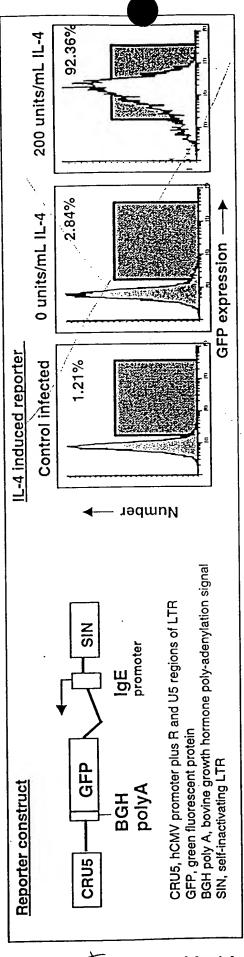


ε heavy chain GFP/BFP knock-in cell line

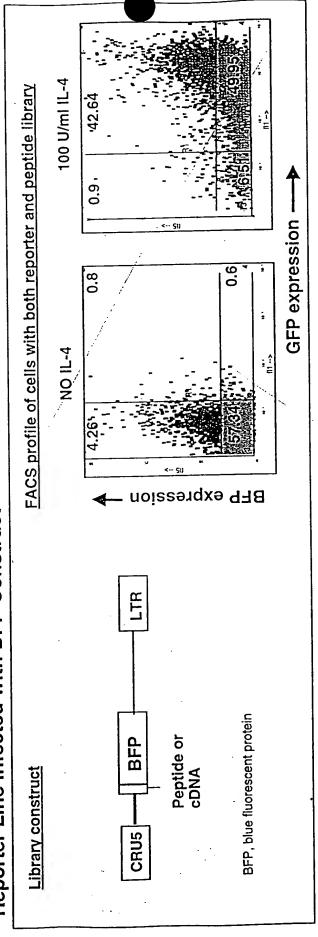


Appendix D

IL-4 Inducible ϵ Promoter Reporter Cell Line



Reporter Line Infected with BFP Construct



Appendix C

Rigel, Inc.

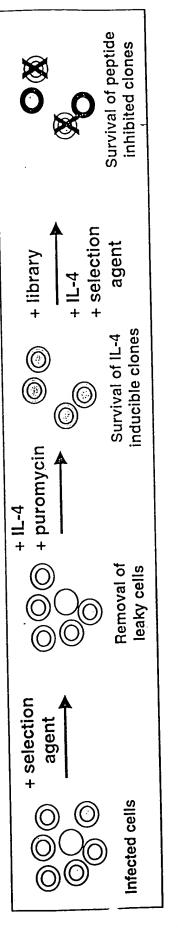
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Confidential

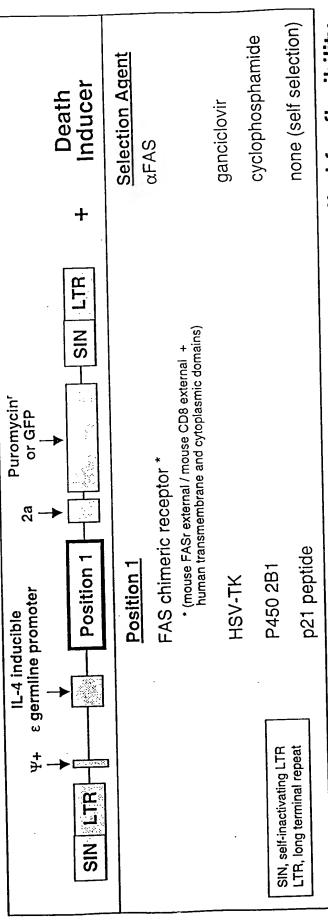
 \bigcirc



Screen for Peptide Inhibitors of the Germline ϵ Promoter



Survival Construct



Appendix D

All components are cassetted for flexibility

FIGURE 11A-1

```
1-845 CMV promoter/R/U5 5' LTR

1322 GAG ATG-ATC mutation

850-2100 extended \psi region

2146-2173 two Bstx1 peptide cloning sites

2205-2723 ECMV IRES (cloned as EcoR1/Msc1 fragment from pCITE-4a [Novagen])

2746-3465 GFP coding region

3522-4115 3' LTR

4122-6210 pGEM backbone (pUC origin, ampR)
```

ATCACGAGGCCCTTTCGTCTTCAAGAACAGCTTTGCTCTTAGGAGTTTCCTAATACATCC CAAACTCAAATATATAAAGCATTTGACTTGTTCTATGCCCTAGTTATTAATAGTAATCAA TTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAA ATGGCCCGCCTGGCTGACCGCCCAACGACCCCCCCCCATTGACGTCAATAATGACGTATG TTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT AAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACG TCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTC AGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCA TTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTA ACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCATGTACGGTGGGAGGTCTATATAA GCAGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACT GAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGT $\tt CTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTT$ CATTTGGGGGCTCGTCCGGGATCGGGAGACCCCTGCCCAGGGACCACCGACCCACCACCG GGAGGTAAGCTGGCCAGCAACTTATCTGTGTCTGTCCGATTGTCTAGTGTCTATGACTGA TTTTATGCGCCTGCGTCGGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGG TGGAACTGACGAGTTCGGAACACCCGGCCGCAACCCTGGGAGACGTCCCAGGGACTTCGG GGGCCGTTTTTGTGGCCCGACCTGAGTCCAAAAATCCCGATCGTTTTGGACTCTTTGGTG CACCCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGACGAGAACCTAAAACAGTTCC GCTGCAGCATCGTTCTGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATA TCGGCCCGGGCCAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGATG TCGAGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTTACCTTCT GCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGCCGCGAGACGGCACCTTTAACCGAG ACCTCATCACCCAGGTTAAGATCAAGGTCTTTTCACCTGGCCCGCATGGACACCCAGACC CCTTTGTACACCCTAAGCCTCCGCCTCCTCTTCCTCCATCCGCCCCGTCTCTCCCCCTTG AACCTCCTCGTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTCACTCCTTCTCTAG GCGCCCCATATGGCCATATGAGATCTTATATGGGGCACCCCCGCCCCTTGTAAACTTCC CTGACCCTGACATGACAAGAGTTACTAACAGCCCCTCTCTCCAAGCTCACTTACAGGCTC TCTACTTAGTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAACTGG AGACTAAGAACCTAGAACCTCGCTGGAAAGGACCTTACACAGTCCTGCTGACCACCCCCA _CCGCCCTCAAAGTAGACGGCATCGCGCTTGGATACACGCCGCCCACGTGAAGGCTGCCGA CCCCGGGGGTGGACCATCCTCTAGACTGCCGGATCTCGAGGGATCCACCACCATGGACCC

FIGURE 11A-2

GGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTG TCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGT CGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGC CACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGA TAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATG CCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACAT GTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCT TTGAAAAACACGATGATAATATGGGGGGATCCACCGGTCGCCACCATGGTGAGCAAGGGCG AGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGA AGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGA CCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCA AGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCA ACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGC TGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACT ACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACT TCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGA ACACCCCATCGGCGACGGCCCCGTGCTGCCCGACAACCACTACCTGAGCACCCAGT CCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGA CCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGCTCGACGA TAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACCTGTA GGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATAACTGA GAATAGAGAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACA GGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTG AATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAA CAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTC CAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCG CTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCC TCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAA ACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGA GTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTCCGACTTGTGGTCTCGCTTGC GAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTCACATGCAGCATGTAT CAAAATTAATTTGGTTTTTTTTTTTAAGTATTTACATTAAATGGCCATAGTTGCATTAAT GAATCGGCCAACGCGCGGGAGAGGCGGTTTGCGTATTGGCGCTCTTCCGCTTCCTCGCT GGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGG CCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCG CCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGG ACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGAC CCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCCTTCGGGAAGCGTGGCGCTTTCTCA TAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGT GCACGAACCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTC CAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAG AGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACAC TAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGT GCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGG GTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAA TATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTC AGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTAC GATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTC ACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGG

FIGURE 11A-3

TCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAG
TAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTC
ACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTAC
ATGATCCCCCATGTTGTGCAAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAG
AAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTAC
TGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTG
AGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAACACGGGATAATACCGC
GCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAAACGTTCTTCGGGGCGAAAACT
CTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTG
ATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAA
TGCCGCAAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTTC
TCAATATTATTGAAGCATTTATCAGGGTTTCTCATGAGCGGATACATATTTGAATG
TATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTC

FIGURE 11B-1

1-845 CMVpormoter/R/U5 5' LTR

1322 GAG ATG-ATC mutation

850-2100 extended [] region

2151-2865 GFP coding region

2866-2894 GGGSGGG linker

2895-2952 FMDV 2a cleavage sequence

2953-3004 Bstx1/Bstx1/HinD3/Hpa1/Sal1/Not1 polylinker

3052-3645 3' LTR

3652-5715 pGEM backbone (pUC origin, ampR)

CCAAACTCAAATATAAAAGCATTTGACTTGTTCTATGCCCTAGTTATTAATAGTAATC AATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGG TAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACG TATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTT ACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTA TTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGG GACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCG GTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTC TCCACCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCA AAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCATGTACGGTGGGA GGTCTATATAAGCAGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTC CTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCA TCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGT CAGCGGGGTCTTTCATTTGGGGGCTCGTCCGGGATCGGGAGACCCCTGCCCAGGGACC TAGTGTCTATGACTGATTTTATGCGCCTGCGTCGGTACTAGTTAGCTAACTAGCTCTGT ATCTGGCGGACCCGTGGTGGAACTGACGAGTTCGGAACACCCGGCCGCAACCCTGGGAG

FIGURE 11B-2

ACGTCCCAGGGACTTCGGGGGCCGTTTTTGTGGCCCGACCTGAGTCCAAAAATCCCGAT CGTTTTGGACTCTTTGGTGCACCCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGA CGAGAACCTAAAACAGTTCCCGCCTCCGTCTGAATTTTTGCTTTCGGTTTGGGACCGAA ${\tt TTTCTGTATTTGTCTGAAAATATCGGCCCGGGCCAGACTGTTACCACTCCCTTAAGTTT}$ GACCTTAGGTCACTGGAAAGATGTCGAGCGGATCGCTCACAACCAGTCGGTAGATGTCA AGAAGAGACGTTGGGTTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGG CCGCGAGACGCCACCTTTAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTTTTC ACCTGGCCCGCATGGACACCCAGACCAGGTCCCCTACATCGTGACCTGGGAAGCCTTGG CTTTTGACCCCCCTCCCTGGGTCAAGCCCTTTGTACACCCTAAGCCTCCGCCTCCTCT CCTCCATCCGCCCGTCTCTCCCCCTTGAACCTCCTCGTTCGACCCCGCCTCGATCCTC CCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCCCCATATGGCCATATGAGATCTTAT AGCCCCTCTCTCCAAGCTCACTTACAGGCTCTCTACTTAGTCCAGCACGAAGTCTGGAG GAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAAGAACCTAGAACCTCGCTGG AAAGGACCTTACACAGTCCTGCTGACCACCCCCACCGCCCTCAAAGTAGACGGCATCGC AGCTTGGATACACGCCGCCCACGTGAAGGCTGCCGACCCCGGGGGTGGACCATCCTCTA GACTGCCGGATCTCGAGGGATCCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGG GTGGTGCCCATCCTGGTCGAGCTGGACGCGACGTAAACGGCCACAAGTTCAGCGTGTC CGGCGAGGGCGAGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCA CCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAG CGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCC GCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATC GACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCA CAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCC GCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCC ATCGGCGACGGCCCCGTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCG CCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGAATTCGGAGGTGGCAGCGGTGGC GGTCAGCTGTTGAATTTTGACCTTCTTAAACTTGCGGGAGACGTCGAGTCCAACCCTGG GCCCACCACCATGGAAGCTTCCATTAAATTGGTTAACGTCGACGCGGCCGCTCGAC GATAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACCT GTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATAA CTGAGAATAGAGAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCC AAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCCAAGAACAGATGGAA CAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGG CCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCA GATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCA ATCAGTTCGCCTTCTGTTCGCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGC CCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCCGT GTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAG GGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTCCGACTTGTGGT CTCGCTGCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTCA CATAGTTGCATTAATGAATCGGCCAACGCGCGGGAGAGGCGGTTTGCGTATTGGCGCT

CTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTA TCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAA GAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGG CGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAG AGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCT ${\tt CGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTT}$ CGGGAAGCGTGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTC GTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTT ATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAG CAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTG AAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCT CAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACG TTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT AAAAATGAAGTTTGCGCAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGT TACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCAT AGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCC CCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATA AACCAGCCAGCCGGAAGGGCCCAGAAGTGGTCCTGCAACTTTATCCGCCTCCAT CCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGC GCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCT TCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAA TATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGA TGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCG ACCGAGTTGCTCTTGCCCGGCGTCAACACGGGATAATACCGCGCCACATAGCAGAACTT TAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCG CTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTT TACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGG GAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTTCCTTTTTCAATATTATTGA AGCATTTATCAGGGTTATTGTCTCATGACATTAACCTATAAAAATAGGCGT

FIGURE 11C-1

1-845 CMVpormoter/R/U5 5' LTR
1322 GAG ATG-ATC mutation
850-2100 extended Tregion
2146-2173 two Bstx1 peptide cloning sites
2173-2214 EoR1/Apa1/Hpa1/Not1 polylinker
2262-2855 3' LTR
2855-4901 pGEM backbone (pUC origin, ampR)

ATCACGAGGCCCTTTCGTCTTCAAGAACAGCTTTGCTCTTAGGAGTTTCCTAATACATCCCAAACTCAAAT ATATAAAGCATTTGACTTGTTCTATGCCCTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAG CCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCG CCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGG TGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATT GACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTG GCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTG CAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCATGT ACGGTGGGAGGTCTATATAAGCAGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTC CGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCT CGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTGGGGGCTC GTCCGGGATCGGGAGACCCCTGCCCAGGGACCACCGACCCACCACCGGGAGGTAAGCTGGCCAGCAACTTA TCTGTGTCTGTCCGATTGTCTAGTGTCTATGACTGATTTTATGCGCCTGCGTCGGTACTAGTTAGCTAACT AGCTCTGTATCTGGCGGACCCGTGGTGGAACTGACGAGTTCGGAACACCCGGCCGCAACCCTGGGAGACGT CCCAGGGACTTCGGGGGCCGTTTTTGTGGCCCGACCTGAGTCCAAAAATCCCGATCGTTTTGGACTCTTTG GTGCACCCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGACGAGAACCTAAAACAGTTCCCGCCTCCG TTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATATCGGCCCGGGCCAGACTGTTACCACTCCCT TAAGTTTGACCTTAGGTCACTGGAAAGATGTCGAGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAG AGACGTTGGGTTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGCCGCGAGACGGCACCTT TAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTTTTCACCTGGCCCGCATGGACACCCAGACCAGG AAGCCTCCGCCTCCTCCTCCATCCGCCCCGTCTCTCCCCCTTGAACCTCCTCGTTCGACCCCGCCTCG ATCCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCCCCATATGGCCATATGAGATCTTATATGGGG CACCCCCCCCCTTGTAAACTTCCCTGACCCTGACATGACAAGAGTTACTAACAGCCCCTCTCTCCAAGCT CACTTACAGGCTCTCTACTTAGTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAACT GGACCGACCGGTGGTACCTCACCCTTACCGAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAAGA ACCTAGAACCTCGCTGGAAAGGACCTTACACAGTCCTGCTGACCACCCCCACCGCCCTCAAAGTAGACGGC ATCGCAGCTTGGATACACGCCGCCCACGTGAAGGCTGCCGACCCCGGGGGTGGACCATCCTCTAGACTGCC GGATCTCGAGGGATCCACCACCATGGACCCCCATTAAATTGGAATTCGGGGCCCAAGCTTTGTTAACGTCG CACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATAACTGAGAA TAGAGAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTA AGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGT GGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGT TTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTA ACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACC CCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTG

FIGURE 11C-2

CAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGC GGGGGTCTTTCATTTCCGACTTGTGGTCTCGCTGCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGT GGCCATAGTTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGCGCTCTTCCGCTT ATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAG GAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATC GACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCC CTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGT GGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTG TGCACGAACCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTA AGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGC TACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGC GGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTC TACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGA TGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCAT AGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAA CGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAG TAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGT TTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAA TATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACT CAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAACACGGGATAAT ACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAG GATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTA CTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAAATGCCGCAAAAAAAGGGAATAAGGGCGACA CGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCAT GACATTAACCTATAAAAATAGGCGT



(1) C12ScFas Survival construct

Cl2ScFas: epsilon-cFas(CD95)-Ires-Hygro-BGH PolyA put into Cl2s vector backwards so that no leaky transcription happens through the cmv promoter. .

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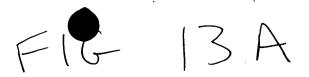
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F16 12B

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ACTG CATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGagtactcaaccaagtcattctgag

aatagtgtatgcggcgaccgagttgctcttgcccggcgtcaacacgggataataccgcgccacatagcagaactttaaaa gtgctcatcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgctgttgagatccagttcgatgtaacccactcgtgcacccaactgatcttcagcatcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatg ccgcaaaaaaggggaataagggcgacacggaaatgttgaatactcatactcttcctttttcaatattattgaagcatttat cagggttattgtctcatgacattaacctataaaaataggcgt



(2) Ahhhh: Survival construct

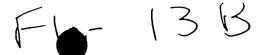
2.) Ahhhh: epsilon-cFas' (CD8 or mLyt2)-Ires-Hygro-BGHpolyA also in C12s backwards

atcacgaggcctttcgtcttcaagaacagctttgctcttaggagtttcctaatacatcccaaactcaaatatataaagc atttgacttgttctatgccctagttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccg cqttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatg ttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggcagta catcaagtgtatcatatgccaagtacgcccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagta catgacettatgggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggc agtacatcaatgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaatgggagtttg ttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcatgt acggtgggaggtctatataagcagagctcaataaaagagcccacaacccctcactcggggcgccagtcctccgattgact qaqtcqcccgggtacccgtgtatccaataaaccctcttgcagttgcatccgacttgtggtctcgctgttccttgggaggg tctcctctgagtgattgactacccgtcagcgggggtctttcatttggggggctcgtccggggatcgggagacccctgcccag ttttatgcgcctgcgtcggtactagttagctaactagctctgtatctggcggacccgtggtggaactgacgagttcggaa cacccggccgcaaccctgggagacgtcccagggacttcgggggccgtttttgtggcccgacctgagtccaaaaatcccga tcgttttggactctttggtgcacccccttagaggagggatatgtggttctggtaggagaagaacctaaaacagttcc tqtctctqtctgactgtgtttctgtatttgtctgaaaatatgggcccgggccagactgttaccactcccttaagtttgac cttaggtcactggaaagatgtcgagcggatcgctcacaaccagtcggtagatgtcaagaagagacgttgggttaccttct gctctgcagaatggccaacctttaacgtcggatggccgcgagacggcacctttaaccgagacctcatcacccaggttaag atcaaggtcttttcacctggcccgcatggacacccagaccaggtcccctacatcgtgacctgggaagccttggcttttga ccccctccctgggtcaagccctttgtacaccctaagcctccgcctctttcctccatccgccccgtctctcccccttg ${\tt aacctcctcgttcgaccccgcctcgatcctccctttatccagccctcactccttctctaggcgcccccatatggccatat}$ gagatettatatggggcacccccgccccttgtaaacttccctgaccctgacatgacaagagttactaacagcccctctct $\verb|ccaagctcacttacaggctctctacttagtccagcacgaagtctggagacctctggcggcagcctaccaagaacaactgg|$ accgaccggtggtacctcacccttaccgagtcggcgacacagtgtgggtccgccgacaccagactaagaacctagaacct cgctggaaaggaccttacacagtcctgctgaccacccccaccgcctcaaagtagacggcatcgcagcttggatacacgc cgcccacgtgaaggctgccgaccccgggggtggaccatcctctagactgccGGATCTCGAGGGATCCTCCCCAGCATGCC

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 $\tt CTGTCTCGACAAGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACTTACCTGCCCAGTGCCTCACG$

AAGATTTTATTTAGTCTCCAGAAAAAAGGGGGGAATGAAAGACCCCACCTGTAGGTTTGGCAAGctagctTAAGTAACCCA TTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGGTCGGAACAGATGGAACAGGCAATAAA AGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCT CTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGG TCTTTCAcatgcaGCATGTATCAAAATTAATTTGGTTTTTTTTTTTTTAAGTATTTACATTAAATGGCCATagtttcGTAAT AAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCT GTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGC GAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCT GGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAG GACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATAC CTGTCCGCCTTTCTCGCGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGT TCGCTCCAAGCTGGGCTGTGCACGAACCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGT CCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGT GCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCAAGCC AGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAC GAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAG TTTGCGCAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCT CAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCA GTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATG





 ${\tt CTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGagtactcaaccaagtcattctgagaatagtgtatgcggcga}$

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